RESEARCH COMMUNICATION

NER and BER Repair Gene Polymorphisms in a Healthy North Indian Cohort and Comparison with Different Ethnic Groups Worldwide

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Abstract

Background: Polymorphisms in DNA repair genes are associated with ability to remove DNA lesions, and therefore may contribute to an individual's susceptibility to different types of cancer. Base excision repair (BER), and nucleotide excision repair (NER) are the main DNA repair pathways. The present study was conducted to determine the frequency distribution of single nucleotide polymorphisms (SNPs) selected for genes in these two pathways i.e. *OGG1* Exon 7 (C1245G), *XPC* Intron 9 (PAT), and Exon 15 (A33512C) in a North Indian population in comparison with global populations. Methods: Genotyping was achieved by PCR-based analysis in 224 normal healthy, unrelated individuals of similar ethnicity. Results: Allelic frequencies in wild type of *OGG1* Exon 7 *C>G* were 73% (C); *XPC* PAT *D>I* 75% (D); and *XPC* Exon 15 *A>C* 60.71.9% A. On the other hand, the variant allele frequency were 27% (G) in *OGG1* Exon 7 *C>G*; 25% (I) in *XPC* PAT; and 28.1% (C) in *XPC* Exon 15 *A>C*. Major differences from other ethnic populations were observed. Conclusions: Our results suggest that frequency distribution in these DNA repair genes exhibited a distinctive pattern in our population which could be attributed to ethnic variation. This could assist in high-risk screening of humans exposed to environmental carcinogens and cancer predisposition in different ethnic groups.

Keywords: DNA repair genes - polymorphisms - ethnic groups

Asian Pacific J Cancer Prev, **11**, 1601-1604

Introduction

Genetic variation in human genome is an emerging resource for studying cancer, a complex disease characterized by both environmental and genetic contributions. Gene-environment interactions may be manifested in various ways, either by risk effects based on an individual's genotype, or differential gene risk effects based on exposure (Vispe et al.,2000).

DNA in most cells is regularly damaged by endogenous and exogenous mutagens. Unrepaired damage can result in apoptosis or may lead to unregulated cell growth and cancer. If DNA damage is recognized by cell machinery, several responses may occur to prevent replication in the presence of genetic errors. At the cellular level, checkpoints can be activated to arrest the cell cycle, transcription can be up-regulated to compensate for the damage, or the cell can apoptose. Alternatively, the damage can be repaired at the DNA level enabling the cell to replicate as planned.

DNA repair mechanisms are varied and complex, At least four important DNA repair pathways, base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR) and double-strand break repair (DSBR), operate on specific types of damaged DNA, and each pathway involves numerous molecules. Several studies while assessing the association of polymorphisms in DNA-repair genes with cancer risk have documented that individuals with ''adverse'' genotypes that result in reduced DRC are at a higher risk of developing cancer than the general population (Wu et al., 2004; Neuman et al., 2005).

We evaluated three polymorphisms in two DNA repair genes: *OGG1* Exon 7 (C1245G), *XPC* Intron 9 (PAT), *XPC* Exon 15 (A33512C) and identified a sufficient number of epidemiologic studies on DNA repair genes to conduct a comparative analysis for genetic polymorphisms in repair pathway genes.

OGG1 encodes 8-hydroxyguanine glycosylase, involved in Base excision repair pathway (BER). Which is assumed to be a key enzyme in the repair of 8-oxo-guanine and other types of oxidative DNA damage (Wang et al., 1995). Polymorphism Ser326Cys is associated with a reduced enzyme activity (Kohno et al., 1998). Since Cys is a low-expression allele for 8-hydroxyguanine glycosylase, *OGG1* Ser326Cys is expected to play a role in human cancer susceptibility.

Xeroderma pigmentosum complementation group C (XPC) is an important DNA damage recognition protein involved in global genome DNA repair (GGR), a subclass of nucleotide excision repair (NER). The *XPC* protein recognizes a variety of bulky DNA adducts formed by

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exogenous carcinogens. It binds tightly with another protein, HR23B, to form a stable *XPC*-HR23B complex. Recent studies identified the *XPC*-HR23B complex as the first protein component that recognizes and binds to the damaged sites. An intronic biallelic poly (AT) insertion/ deletion polymorphism (*XPC* PAT) of the XPC gene consisting of an insertion of 83 bases of A and T [poly (AT)] and deletion of 5 bases (GTAAC) at positions 1457 to 1461 in intron 9 has been reported. *XPC*-PAT polymorphism has been reported to be in linkage disequilibrium with a single nucleotide polymorphism in *XPC* Exon 15 that causes an amino acid change Lys939Gln (A33512C, rs2228001) has also been reported. Interestingly, defects in XPC have been associated with many types of cancer (Wang et al., 2003).

The present study is an attempt to investigate frequency distribution of *OGG1*, *XPC* genes polymorphism by using a PCR-based restriction analysis in unrelated normal healthy individuals from North India.

Materials and Methods

Subjects

The study involved 224 subjects from the North Indian population, which included unrelated healthy subjects from same geographical region. The hospital ethics committee approved the study and informed consent was obtained from the participating volunteers.

DNA Extraction

Five ml of blood was collected in EDTA vials and DNA was extracted from blood lymphocytes using 'salting out' method (Miller et al., 1998).

Table 1. Genotypes and Allele Frequency Distribution of OGG1 Exon 7, XPC Pat And XPC Exon 15 Gene Polymorphism in North India

Gene	Geno-type	Observed (n) %	Expected (n) %	Minor allele freq	P-value (HWE)
OGG1 Exon 7	CC	116 (51.8)	119 (53.3)	27	0.25
C1245G	CG	95 (42.4)	89 (39.4)		
(rs1052133)	GG	13 (5.8)	16 (7.3)		
XPC Intron 9	D/D	124 (55.3)	126 (56.2)	25	0.47
PAT	D/I	88 (39.3)	84 (37.5)		
(AF076952)	I/I	12 (5.4)	14 (6.3)		
XPC Exon 15	AA	114 (50.9)	116 (51.7)	28	0.56
A33512C	AC	94 (42.0)	91 (40.4)		
(rs2228001)	CC	16 (7.1)	17 (7.9)		

Genotyping

All study samples were genotyped for the three SNPs in two DNA repair genes that included *OGG1* Exon 7 (rs1052133; C1245G), *XPC* Intron 9 (AF076952; PAT), *XPC* Exon 15 (rs2228001; A33512C), using PCR-RFLP (restriction fragment length polymorphism method) and ARMS-PCR (Amplification Refractory Mutation System) method as described earlier (Le Marchand et al., 2002; Lopez cima et al., 2007). Positive and negative controls were used in each genotyping assay, and 5% of the samples were randomly selected and run in duplicates with 100% concordance. The results were reproducible with no discrepancy in genotyping.

Prevalence of gene variants

We conducted a MEDLINE search using "*OGG1*", "*XPC*", "polymorphism" for papers published before December 2009. The search was limited to human subjects, without language restriction. For case-control studies, only genotype frequencies for the control population were considered. Studies that reported only allele frequencies and no genotype frequencies were not included. Studies based on fewer than 90 persons were excluded. The most recent publication was included in the study when more than one article was identified for the same study population. We identified 9 publications reporting on the prevalence of *OGG1* Exon 7 polymorphism, 4 publications on *XPC* PAT and 3 for *XPC* Exon 15 which were subsequently used for comparison with our study.

Statistical analysis

Pearson's χ^2 test was done to compare the genotype and allelic frequencies of different populations using the software SPSS (version 11.5). Court-Lab (web-based software) was used to examine Hardy-Weinberg equilibrium (www.tufts.edu). P-value 0.05 was considered to be statistically significant.

Results

The distribution of *OGG1* Exon 7, *XPC* PAT, and Exon 15 genotypes and allele frequencies in northern Indian population are shown in Table 1. Genotype distributions were in agreement with Hardy-Weinberg equilibrium for all the 3 SNPs selected.

The genotypic and allelic frequencies of the three genes in different populations with reference to our

Table 2. Genotypes and Allele Frequency Distribution of OGG1 Exon 7 Gene Polymorphism in Various Populations and P-value in Comparison to North Indian Population

Gene	Country/ ethnicity	n	Age (years), Mean age ± SD	Genotype CC	CG	GG	P value	G	Ref
OGG1 Exon 7	North India	224	59.1 ± 10.4	116 (51.8)	95 (42.4)	13 (5.8)	Ref	27	Present Study
	Japan	121	67.4 ± 6.7	39 (32.2)	54 (44.6)	28 (23.2)	<0.001	45.5	Miyaishi et al., 2009
	Spain	323	-	210 (64.9)	104 (32.3)	9 (2.8)	0.033	18.9	Nock et al., 2006
	Norway	386	60(50-85)	194 (50.3)	117 (30.3)	75 (19.4)	<0.001	34.6	Zienolddiny et al., 2006
	Minnesota	599	59.7 ± 12.1	339 (57)	223 (37)	37 (6)	0.938	24.8	McWilliams et al., 2008
	Korea	247	-	52 (21.1)	131 (53.0)	64 (25.9)	<0.001	52.4	Kim et al., 2003
	Turkey	250	53.19 ± 0.75	115 (46)	106 (42.4)	29 (11.6)	0.024	32.8	Karahalil et al., 2008
	USA	479	62.8 ± 9.1	305 (63.8)	142 (29.7)	32 (6.5)	0.849	21.5	Nock et al., 2006
	Polish	100	-	68 (68)	28 (28)	4 (4)	0.276	18	Sliwinski et al., 2009

Table 3. Genotypes and Allele Frequency Distribution of XPC PAT Gene Polymorphism in Various Populations and P-values in Comparison to Northern Indian Population

	Country/ ethnicity	n	Age(years), Mean age ± SD	Genotypes D/D	D/I	I/I	P value	I	Reference
XPC PAT	North India	224	59.1 ± 10.4	124 (55.3)	88 (39.3)	12 (5.4)	Ref	25	Present Study
	USA	562	63.1 ± 10.5	214 (38.1)	258 (45.9)	90 (16)	<0.001	39	Zhu et al., 2007
	Spain	375	62.94 ± 11.81	132 (35.2)	190 (50.7)	53 (14.1)	<0.001	39.5	Marin et al., 2004
	Canada	95	-	41 (43)	42 (44)	12 (13)	0.029	34.7	Casson et al., 2005

Table 4. Genotypes and Allele Frequency Distribution of XPC Exon 15 Gene Polymorphism in Various Populations and P-values in Comparison to Northern Indian Population

Gene	Country/ ethnicity	n	Age(years), Mean age ± SD	Genotypes AA	AC	CC	P value	C	Reference
XPC Exon 15	North India	224	59.1 ± 10.4	114 (50.9)	94 (42.0)	16(7.1)	Ref	28.1	Present Study
	Texas	220	49.3 ± 15.2	79(36)	97(44)	44(20)	<0.001	42	El-Zein et al., 2009
	Minnesota	598	59.7 ± 12.1	221 (37)	285(48)	92(15)	<0.001	39.2	McWilliams et al., 2008
	USA	165	67 ± 15	72(44)	70(42)	23(14)	0.022	35	Hirata et al., 2007

population were compared using χ^2 test (Tables 2, 3 and 4). The minor allele frequency in our population was 27%, 25% and 28% for *OGG1* Exon 7, *XPC* PAT and *XPC* Exon 15 respectively.

In case of *OGG1* Exon 7 (C1245G) significant frequency distribution was observed in Japan, Spain, Norway and Korea as compared to our population. Significantly different pattern of genotype and allele frequencies was reported in *XPC* PAT polymorphism in USA, Spain and Canada population. Genotype and allele distribution of *XPC* Exon 15 (A33512C) polymorphism was significantly different from Texas, Minnesota and USA.

Discussion

Functional polymorphisms of genes for DNA repair are of particular importance from the point of view that they are implicated in the pathogenesis of complex genetic disorders. A number of studies suggest that such mild defects in DNA repair may predispose to cancer (Au et al., 2004). Due to marked differences in the distribution of DNA repair polymorphisms, between various worldwide ethnic groups, the data from 'normal healthy' populations are of special interest for finding out the relevance as well as the evaluation of the investigated genetic markers in susceptibility, manifestation, prognosis or treatment of diseases. It is well recognized that ethnic background may influence the susceptibility to suffer from certain diseases (Kittles et al., 2003). Therefore, variation in our Indian population in contrast to other populations worldwide signifies the impact of ethnicity. Indian population is believed to be most diverse because of different socio-cultural traditions. The study of genetic variation can elucidate critical determinants in environmental exposure and cancer, which could have future implications for preventive and early intervention strategies. The differences in the allelic frequencies detected among these studies might be due to several reasons such as ethnic variation, heterogeneity of study populations and different sample sizes.

In *OGG1* Exon 7 (C1245G) polymorphism, the (G*) allele frequency in Indian population was 27%, which was significantly higher in Japan, Spain, Norway, Korea and Turkey and no significant difference was observed from Minnesota, USA and Polish. The (I*) allele frequency in XPC PAT polymorphism was 25% in our population. This was significantly lower as compared with that of USA, Spain and Canada. In *XPC* Exon 15 (A33512C) polymorphism, the (C*) allele frequency in Indian population was 28.1% which was significantly higher as compared to observed in populations from Texas, Minnesota and USA.

In a study from Minnesota and Turkey by McWilliams et al., 2008, and Karahalil et al., 2008, the minor variant allele frequencies were found to be almost similar with our northern population for *OGG1* Exon 7 (27% vs. 24.8% and 32.8%) respectively.

Although the increased/decreased risk associated with individual DNA repair SNPs may be small compared to that conferred by high-penetrance cancer genes, their public health implication may be large because of their high frequency in the general population. Epidemiological investigations of DNA repair polymorphisms are therefore important (Wacholder et al., 2004). Large and combined analyses may be preferred to minimize the likelihood of both false-positive and false-negative results. Appropriate, confounding factors should be controlled, in particular consideration of race and ethnicity. As there are differences in the prevalence of DNA repair polymorphisms across different populations, hence, it is important to keep in mind that a susceptibility factor in one population may not hold true for another. Such kind of study may form the basis for future establishment of epidemiological and clinical databases. The present analyses suggest that *OGG1* and *XPC* polymorphisms may be biomarkers of disease susceptibility and may be contributing factors in the risk of cancer development. A single larger study with thousands of subjects and tissue-specific biochemical and biological characterization is warranted to further evaluate potential gene-to-gene and gene-to-environment interactions on DNA repair polymorphisms and cancer risk. The differences in these genes distribution between North Indian healthy population and other ethnic groups may help in building a profile that would help in assessing the disease predisposition and prevalence.

Acknowledgments

The authors are grateful to the Director and Head of the Department, Urology, Sanjay Gandhi Post Graduate Institute of Medical Sciences for providing the necessary facilities. RKM thanks the Council of Scientific and Industrial Research New Delhi for awarding a junior research fellowship.

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